

GeneDex

Editor: Michele Nealen

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No matter why you're doing transfections, **TurboFectin 8.0** is the reagent for you

Transfection reagents have evolved since the early days of calcium phosphate-precipitated DNA slamming its way into cells. Now transfection reagents include highly sophisticated compounds that are optimized for maximum transfection efficiency for each type of nucleic acid, and minimal toxicity to the target cells.

Today's chemical transfection reagents are commonly liposomal formulations or lipid and protein combinations. Liposomal reagents penetrate mammalian cell walls efficiently, but often demonstrate high toxicity due to this enhanced ability to enter the cells. The lipid/protein combinations are slower to penetrate cell walls, but offer excellent efficiency coupled with reduced toxicity. OriGene's TurboFectin 8.0 is a lipid/histone formulation that is ideal for most transfection experiments. Regardless of the purpose of your experiment or the type of cell you're transfecting, your experiment can benefit from using TurboFectin 8.0. But don't just take our word for it, see for yourself!

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PROTEIN PRODUCTION

If the purpose of your experiment is to maximize protein production from mammalian cells, it is important to choose a low toxicity/high efficiency reagent that works well in a transfected cell line such as HEK293T cells. OriGene's data indicate that a competitor's reagent shows slightly higher levels of protein activity on day one, but cells transfected with TurboFectin 8.0 are producing significantly more active protein at 48 and 72 hours post-transfection (Figure 1).

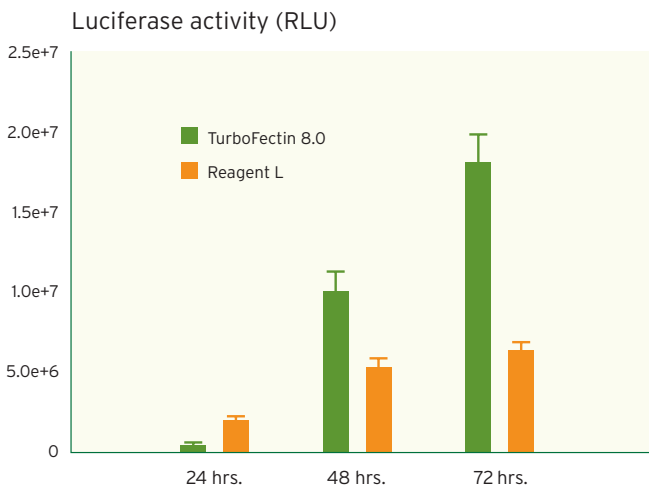


Figure 1. Optimal protein expression over time. HEK293T cells were plated at 10,000 cells/well in a 96-well plate in DMEM with 10% FBS. Cells were transfected with pCMV-Luciferase using the optimal concentration of TurboFectin 8.0 or Reagent L. Luciferase activity was measured at 24, 48, and 72 hours using BriteLite substrate (Perkin Elmer). Results are shown as an average of triplicate wells with standard deviation.

PHENOTYPE ANALYSIS

If you're planning to overexpress or silence your favorite gene then analyze the transfected cells for changes, it's important to maximize the number of successfully transfected cells and minimize toxicity over time. The longer your cells can be studied under the altered condition, the more data can be acquired, including phase contrast and fluorescent images, growth curves, and alterations in expression levels of downstream targets. TurboFectin 8.0 extends the duration of your experiments so that you can maximize data acquisition from every transfection. It is even gentle enough to be used with primary cells. The data provided in Figure 2 demonstrate the high expression level and low mortality rate of primary human chondrocytes transfected with a GFP reporter plasmid using TurboFectin 8.0 and a competitor's reagent.

TurboFectin 8.0 extends the duration of your experiments so that you can maximize data acquisition from every transfection. It is even gentle enough to be used with primary cells.

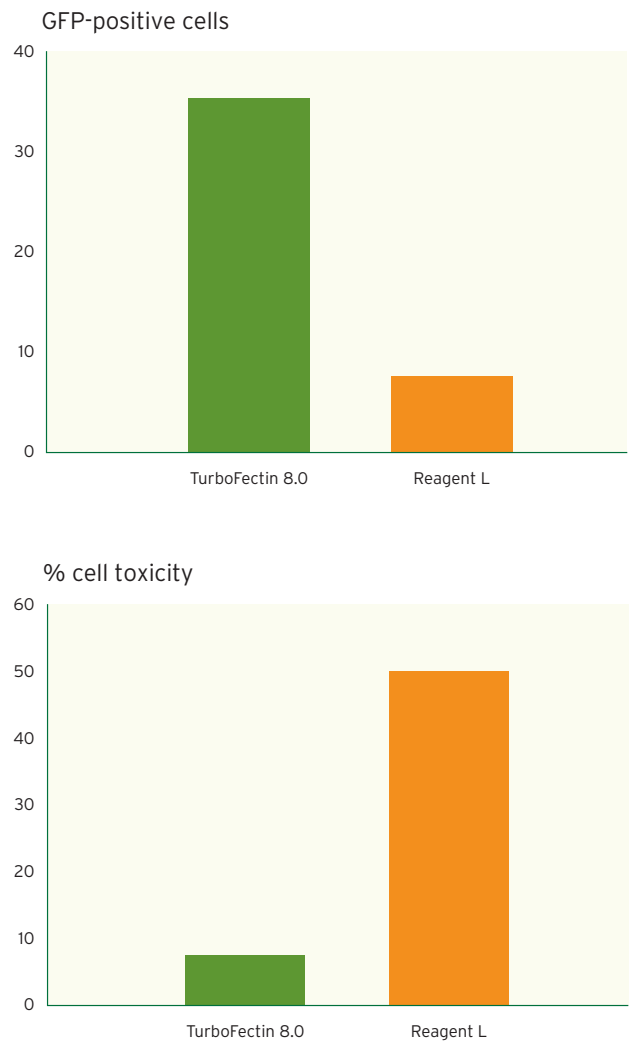


Figure 2. High potency and low toxicity are key features of TurboFectin 8.0 transfections. Primary human chondrocytes were plated at 100,000 cells/well in a 24-well plate and transfected with 500 ng of pGFP using the optimal ratio of TurboFectin 8.0 or a leading transfection reagent. Potency (top panel) and toxicity (bottom panel) were determined by counting viable GFP-positive cells/field at 48 hours post-transfection. Data provided courtesy of David Hum via CedarLane Laboratories.

SILENCING

If you're planning an RNA interference experiment, your choice of transfection reagent will depend on whether you're planning to deliver an shRNA plasmid or siRNA oligonucleotides.

Due to the specific composition of TurboFectin 8.0, this reagent is not recommended for siRNA applications. However, our data indicate that TurboFectin 8.0 is highly effective at delivering shRNA plasmids and can provide double the knock-down compared to the leading reagent.

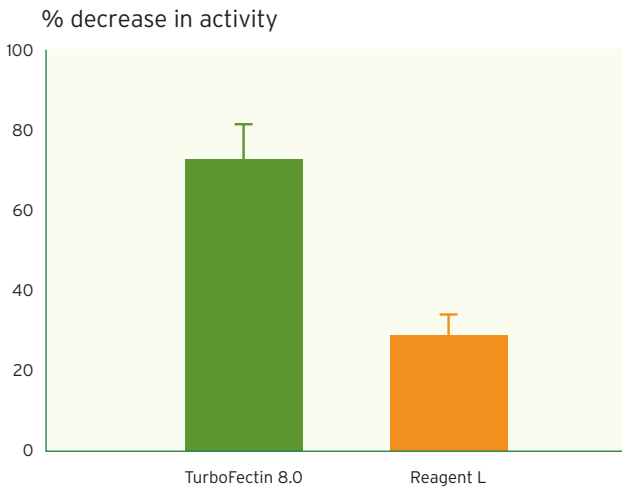


Figure 3. RNAi experiments using shRNA plasmids are more effective when using TurboFectin 8.0 for transfection. HEK293T cells were plated at 10,000 cells/well in a 96-well plate in DMEM with 10% FBS. pCMV-luciferase and the HuSH-29 construct shLuc were transfected using the optimal ratio of DNA to transfection reagent for each product. Luciferase activity was measured at 48 hours using BriteLite substrate (Perkin Elmer). Results are shown as the average percent silencing in triplicate experiments, with standard deviation.

Drive your transfections full throttle with the high performance reagent TurboFectin 8.0. No matter the purpose of your transfection experiment, you'll be rewarded with a more robust outcome.

ORDERING INFORMATION

TurboFectin 8.0 (1 vial @ 1ml each)

TF81001 \$270 USD

TurboFectin 8.0 (5 vials @ 1ml each)

TF81005 \$1,080 USD

For more information on TurboFectin 8.0, please visit our website at: <http://www.origene.com/cdna/turbofectin.msp> or call Technical Support at 888-267-4436 or 301-340-3188.

Want to know how much your data is worth to us?

Let us know how you'd like to use an OriGene product to further your research, and OriGene may make that possible...for free!

We have several programs available to collaborate with scientists on research projects that would benefit from using HuSH-29 constructs, GFC-Transfection Arrays, TissueScan Oncology Panels, or other OriGene products. If you are interested in such a collaboration, and would like to receive free clones, arrays, HuSH constructs or other reagents, send an email to cdna@origene.com with the subject line "collaboration." One of our highly trained scientists will contact you to discuss possible joint research ventures and the delivery of free OriGene products.



October's Trivia Question

Which human chromosome contains the most (currently identified) genes of known sequence?

Send your answer to cdna@origene.com. The first ten correct responses received will win free OriGene merchandise, including an OriGene tote. Please include your full mailing address with your response.

TurboFectin 8.0 Protocols

TRANSFECTION OPTIMIZATION

Although the transfection protocols below have been proven to result in highly efficient transfection, it is important to carefully optimize the reaction conditions for each cell type transfected. The following variables should be considered:

Cell density (percent confluence at transfection)

The recommended confluence for most cell types at transfection is 50–70%. Determine the optimal cell density for your cell type for maximal efficiency and maintain that density in all experiments.

Media conditions TurboFectin 8.0 performs better in complete growth medium compared to serum-free medium. No media change is necessary before or after the transfection.

DNA purity and concentration OriGene recommends highly purified, sterile DNA prepared on ion-exchange columns. It is important to remove contaminating endotoxins from the DNA prep for maximal transfection efficiency.

TurboFectin 8.0 to DNA ratio The standard ratio is 3 μL of TurboFectin 8.0 to 1 μg of DNA in HEK293 cells. It is recommended to titrate the ratio by adjusting the volume of TurboFectin 8.0 from 2–8 μL per 1 μg DNA. Refer to Table 1 for suggested masses and volumes, and remember to optimize for your particular cell type.

Table 1. Recommended starting transfection conditions for TurboFectin 8.0

Culture Plate	10 cm dish	6-well	12-well	24-well	48-well	96-well
surface area (cm^2)	59	9.6	3.8	1.9	1.0	0.35
volume of complex (μL)	600	100	50	20	10	5
TurboFectin 8.0 (μL)	18	3	1.5	0.6	0.3	0.15
DNA (μL ; 1 $\mu\text{g}/\mu\text{L}$ stock)	6	1	0.5	0.2	0.1	0.05

PROTOCOL FOR TRANSIENT TRANSFECTION (ADHERENT CELLS)

A sample protocol is listed here for experiments performed in 6-well plates. If performing experiments in other cell culture vessels, simply multiply the suggested quantities by the relative surface area of your plate (see Table 1).

1. Cell Plating

- On the day before transfection, plate cells at a density of $1-3 \times 10^5$ cells per well in complete growth medium to obtain 50–70% confluence on the following day.
- Incubate overnight.

2. Complex formation (perform this step immediately before transfection)

- In a sterile plastic tube, add 100 μL of serum free medium.
- Add the appropriate amount of TurboFectin 8.0 (2–6 μL per 1 μg DNA) into tube. Mix completely by gentle pipetting.
- Incubate at room temperature for 5 minutes.
- Add plasmid DNA (1–3 μg per well) to the TurboFectin 8.0-containing media prepared above. Mix by gentle pipetting.
- Incubate at room temperature for 15–30 min.

3. Transfection

- If necessary, remove spent medium from the cells prepared in Step 1 and replace with 2 mL of fresh complete medium per well.
- Add the mixture prepared in Step 2 dropwise to the cells. Gently rock the dish to distribute the complex evenly.
- Incubate for 24–48 hours.

Note: With TurboFectin 8.0, no medium change is necessary. If you wish to remove the complex, remove the medium 4–24 hours post-transfection and replace with fresh complete medium.

PROTOCOL FOR TRANSIENT TRANSFECTION (SUSPENSION CELLS)

A sample protocol is listed here for experiments performed in 6-well plates. If performing experiments in other cell culture plates, simply multiply the suggested quantities by the relative surface area of your plate.

1. Prepare cells for transfection

- On the day before transfection, plate your target cells at a density of $5-10 \times 10^5$ cells per well in complete growth medium for optimal cell density on the following day.
- Incubate overnight.

2. Combine DNA and transfection reagent

- In a sterile plastic tube, add 250 μ L of serum free medium.
- Add the appropriate amount of TurboFectin 8.0. Mix completely by gentle pipetting.
- Incubate at room temperature for 5 minutes.
- Add plasmid DNA to the TurboFectin 8.0-containing media prepared above. Mix by gentle pipetting.
- Incubate at room temperature for 15–30 min.

3. Transfect cells

- If necessary, spin down the cells prepared in Step 1 and remove spent medium. Resuspend the cells in 2.5 mL of fresh complete medium per well.
- Add the mixture prepared in Step 2 dropwise to the cells. Gently rock the dish to distribute the complex evenly.
- Incubate for 24–48 hours.

Note: With TurboFectin 8.0, no medium change is necessary. If you wish to remove the complex, remove the medium 4–24 hours post-transfection and replace with fresh complete medium.

PROTOCOL FOR STABLE TRANSFECTION

Perform a transient transfection as described above. Twenty-four hours post-transfection, passage the cells (at 1:10 or higher dilution) into fresh growth medium containing the selection agent. A mock selection plate (containing untransfected cells, or cells transfected with an antibiotic sensitive plasmid) should be prepared in parallel as a negative control.

PROTOCOL FOR REVERSE TRANSFECTION

In a conventional or “forward” transfection, the cells are grown in the tissue culture plate before the DNA and transfection reagent are applied. In a reverse transfection, the plasmid DNA is deposited in the plate before the transfection reagent or cells are added. Reverse transfection is usually utilized in high-throughput applications when many individual plasmid DNAs are arrayed onto a matrix, such as a glass slide, or in a multi-well plate. For example, in OriGene’s GFC-Transfection Arrays, hundreds to thousands of individual cDNAs are arrayed into 96 or 384-well plates and lyophilized. The target cells and reporter plasmid are then added to these wells by the end user. TurboFectin 8.0 is the preferred reverse transfection reagent for GFC-Transfection Arrays.

For different cell lines, the optimal ratio of TurboFectin 8.0 (μ L): DNA (μ g) may be quite different. Three ratios are recommended for initial testing: 3:2, 3:1, and 6:1. The protocol below describes the application using OriGene’s standard GFC-Trans-

fection Array in 384-well plates (60 ng DNA per well) and using a TurboFectin 8.0:DNA ratio (μ L: μ g) of 3:1. For 96-well plates, simply substitute the recommended volumes from Table 2.

Table 2. Recommended starting transfection conditions for reverse transfection protocols using multi-well plates

Contents (per well)	96-well	384-well
plasmid DNA (ng)	100–125	30–60
TurboFectin 8.0 (μ L)	0.3–0.38	0.09–0.18
complex volume (μ L)	10–20	10
number of cells	10,000–20,000	5,000–7,500
final volume (μ L)	up to 200	up to 60

- Equilibrate the sealed plates and serum-free medium at room temperature. If a reporter plasmid is needed, add the reporter to 4 mL of serum free medium. Dispense 10 μ L of the reporter/medium into each well using a multi-channel pipettor. Shake the plate on a microplate mixer for 10 minutes and collect the solution by brief centrifugation.
- For each 384-well plate, prepare 4 mL of serum-free medium in a sterile tube. Add 80 μ L of TurboFectin 8.0 into the medium and mix thoroughly by pipetting up and down.
- Dispense 10 μ L of the diluted transfection reagent into each of the wells using a multi-channel pipettor. Shake on a microplate mixer for 30 seconds and then centrifuge briefly to collect the solution.
- Incubate at room temperature for 20–45 minutes to allow DNA/TurboFectin 8.0 complex formation.
- Prepare the cells to be transfected. If using adherent cells, remove the cells from the growth surface by trypsinization, neutralize the solution, and gently pellet the cells. If using suspension cells, simply pellet the cells. Resuspend the cells in pre-warmed complete media, determine the cell concentration, and adjust the cell density to about 250,000 cells/mL with complete medium.
- Aliquot 30 μ L of cell suspension into each well with a multi-channel pipettor. Let cells settle by gravity. Do not vortex, mix or spin down the cells (unless required by the assay).
- Cover the plate and incubate at 37°C in a CO₂ incubator for 48 hours.

Frequently Asked Questions...

about TurboFectin 8.0

WHAT KIND OF REAGENT IS TURBOFECTIN 8.0?

Answer: TurboFectin 8.0 is a proprietary mixture of a broad-spectrum protein/polyamine with histones and lipids.

WHAT IS THE ADVANTAGE OF TURBOFECTIN 8.0 OVER OTHER TRANSFECTION REAGENTS?

Answer: TurboFectin 8.0 produces lower toxicity and higher expression levels in the most commonly used cell lines than comparable reagents from other commercial sources. Please see the data comparing transfection reagents in HEK293 cells presented on our website at <http://www.origene.com/cdna/turbofectin.msp>.

WHAT TYPES OF MOLECULES CAN BE TRANSFECTED USING TURBOFECTIN 8.0?

Answer: TurboFectin 8.0 is recommended for transfecting DNA, including cDNA expression plasmids and shRNA constructs. You can perform cotransfections of multiple plasmids, as well as either forward or reverse transfections. At this time, we do not recommend using TurboFectin 8.0 to transfect siRNA or oligonucleotides.

HAS TURBOFECTIN 8.0 BEEN TESTED ON TRANSFORMED CELL LINES AND PRIMARY CELLS?

Answer: Yes, it has. You can find the current list of primary cells and cell lines that have been successfully transfected with TurboFectin 8.0 on our website at http://www.origene.com/assets/Documents/Transfection/TurboFectin8_cellsline.xls.

MY CELLS ARE GROWN IN SERUM-FREE MEDIUM. CAN I STILL USE TURBOFECTIN 8.0 TO TRANSFECT THEM?

Answer: Yes. While TurboFectin shows the best transfection efficiency in medium containing serum, it is also effective at transfecting cells grown in medium that does not contain serum.

WILL THE USE OF ANTIBIOTICS IN MY GROWTH MEDIUM INTERFERE WITH TRANSFECTION EFFICIENCY?

Answer: While TurboFectin 8.0 has been shown to be effective in the presence of antibiotics, if you have low transfection efficiency you may wish to test the protocol again in the

absence of antibiotics. Certain cationic antibiotics (such as kanamycin) may decrease the efficiency of TurboFectin 8.0 transfection. Even though penicillin and streptomycin are not toxic to eukaryotic cells, during transfection the cell permeability increases so that much higher levels of antibiotics can enter the cells, which can increase toxicity.

WHY CAN'T I PERFORM A TRANSFECTION ON CELLS THAT ARE LESS THAN 70% CONFLUENT?

Answer: Cell density can affect transfection efficiency. We recommend transfecting cells that have been passaged the previous day (for adherent cells) and have grown to 50–70% confluency.

HOW LONG DO I NEED TO LEAVE THE TURBOFECTIN 8.0 – DNA SOLUTION ON MY CELLS BEFORE ASSAYING THEM?

Answer: We recommend a minimum of 24 hours of incubation prior to assay. Depending on your assay, a longer time (48–72 hours) may be required to produce the necessary effect, but the transfection solution can be removed from the cells after 24 hours if necessary. However, a medium change is not necessary, so the transfection solution can remain on the cells for the full incubation period.

WHAT IS THE STABILITY OF THIS TRANSFECTION REAGENT?

Answer: TurboFectin 8.0 is shipped on wet ice and should be stored at 4 °C. If stored and handled properly, this reagent has a shelf life of twelve months after purchase. It is important not to freeze the reagent, as this will cause decreased efficiency of transfection.

WHY HAS MY TURBOFECTIN 8.0 DEVELOPED A PRECIPITATE DURING STORAGE?

Answer: Storage at -20°C may cause some precipitation in the TurboFectin, but this is completely normal and reversible. Simply warm the reagent to room temperature and gently vortex before use. You may store TurboFectin at +4°C to avoid the development of precipitates.

TurboFectin 8.0 is a proprietary mixture of a broad-spectrum protein/polyamine with histones and lipids.

WHY DO I NEED TO OPTIMIZE THE RATIO OF TURBOFECTIN 8.0 TO DNA FOR EVERY CELL LINE I TRANSFECT?

Answer: Every cell line has different cellular dynamics, which affect transfection efficiency. Therefore, we recommend a range of ratios of transfection reagent to DNA that work well in a number of commonly used cell lines. We recommend using multiple ratios when beginning your work with TurboFectin 8.0, and determining the best ratio of transfection reagent to DNA for your cell line. If you need assistance with this optimization, please contact our Technical Support Specialists for suggestions.

WHAT FACTORS WILL AFFECT THE TRANSFECTION EFFICIENCY OF A TURBOFECTIN 8.0 EXPERIMENT?

Answer: Many aspects of the transfection complex formation and culturing of the transfected cells can negatively affect the efficiency of transfection with TurboFectin 8.0. Using medium containing serum to complex DNA with TurboFectin 8.0, using a suboptimal ratio of DNA to transfection reagent, using impure DNA or cells that are less than 50% confluent can all decrease the transfection efficiency.

WHAT FACTORS WILL AFFECT THE CYTOTOXICITY OF CELLS TRANSFECTED WITH TURBOFECTIN 8.0?

Answer: Similarly, many aspects of transfection complex formation and culturing of the transfected cells can exaggerate the toxic effects of transfection. Using a suboptimal ratio of DNA to transfection reagent, using impure DNA or cells that are less than 50% confluent can all increase toxicity in a transfection experiment.

WHY DO YOU RECOMMEND THAT THE DNA USED IN TRANSFECTIONS SHOULD BE ENDOTOXIN-FREE?

Answer: Some cells are particularly sensitive to endotoxins, and will show a high level of mortality if transfected with DNA that is not endotoxin-free. Many commercially available DNA purification kits such as Qiagen's QIAprep Spin Miniprep Kit or Marligen's Rapid Plasmid Miniprep Purification System will produce endotoxin-free DNA from bacterial cultures.

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In the coming weeks, look for the launch of the TrueORF product line. Each of these cDNA clones contains only the open reading frame (ORF) of a TrueClone, trimmed to remove the 5' and 3' untranslated regions (UTRs). Each fully sequenced ORF has been cloned into the TrueORF Entry Vector. This construct can be directly used for transient or stable transfection of cultured mammalian cells or for in vitro protein expression. The ORF can be easily and directionally subcloned into one of many available destination vectors containing N-terminal or C-terminal tags of HA, His, MYC, FLAG, or GFP. So if you want to do more with your human cDNA expression clone, look for the TrueORFs from OriGene!

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Genes in the News

FOXP3 IS AN X-LINKED BREAST CANCER SUPPRESSOR GENE AND AN IMPORTANT REPRESSOR OF THE HER-2/ERBB2 ONCOGENE

Tao Zuo, Lizhong Wang, Carl Morrison, Xing Chang, Huiming Zhang, Weiquan Li, Yan Liu, Yin Wang, Xingluo Liu, Michael W.Y. Chan, Jin-Qing Liu, Richard Love, Chang-gong Liu, Virginia Godfrey, Rulong Shen, Tim H.-M. Huang, Tianyu Yang, Bae Keun Park, Cun-Yu Wang, Pan Zheng, and Yang Liu

Cell 129, 1275-1286, June 29, 2007

While significant advances have been made in understanding the mechanism of breast cancer, there are many more questions still unanswered. Oncogenes remain to be identified, and the regulators of these oncogenes (potential tumor suppressor genes) have not been sufficiently described either. The gene ERBB2 (also known as HER-2 or Neu) was identified as a breast cancer oncogene in 1984 (Schechter et al.) and expression levels of this gene are often used to predict clinical outcomes in patients with breast cancer. However, the tumor suppressor genes that keep ERBB2 expression in check in normal tissue have not been identified. Although gene amplification can lead to ERBB2 overexpression (Slamon et al.), many patient samples with increased levels of ERBB2 expression lack gene amplification (Bofin et al.), implying that gene upregulation is a potential and undescribed mechanism of ERBB2 overexpression in breast cancer.

FOXP3, a gene located on the X chromosome, encodes a transcription factor essential for normal T cell development (Hori et al., Fontenot et al. 2003, Fontenot et al. 2005). During studies involving position cloning of a gene responsible for X-linked autoimmune diseases, researchers from the University of Michigan, Ohio State University, and the University of North Carolina at Chapel Hill noticed that nearly 90% of female mice with only one copy of the FOXP3 gene developed spontaneous malignant tumors, approximately 60% of which were mammary carcinomas. Forty percent of these cancers were metastatic.

Laser capture microdissection coupled with real time PCR analysis was used to measure FOXP3 expression levels in normal mammary epithelium and cancerous cells from these mice. The FOXP3 transcript was detectable in normal cells but completely undetectable in cancer cells. Immunohistochemical analysis confirmed these results.

Although FOXP3 had not been previously implicated in breast cancer, the high incidence of tumor development in FOXP3 mutant mice and the lack of FOXP3 expression in cancerous cells predicted that FOXP3 might be a novel tumor suppressor gene. Conjecturing that FOXP3 could be regulating a common breast cancer oncogene, the researchers looked for effects of mutant FOXP3 on mouse ErbB2. Mammary tumors from the FOXP3 mutant mice showed increased levels of ErbB2 expression. Real time PCR analysis indicated an 8-12 fold increase in ErbB2 transcripts in cancer cells relative to the normal mammary epithelium from these mice. As this data supports the theory that FOXP3 is a tumor suppressor of ErbB2, the scientists looked to demonstrate this effect in a cell culture system. The cDNA of FOXP3 was overexpressed in the TSA cell line, resulting in a decrease in the expression level of ErbB2. EMSA data indicated significant binding of FOXP3 to the promoter region of the ErbB2 gene. Deletion of two potential binding sites in the ErbB2 promoter region substantially increased expression from the ErbB2 promoter, indicating an escape from FOXP3 repression.

Two common somatic mutants of FOXP3 showed decreased repressor activity on ERBB2 in promoter studies. This decrease in repression was accompanied by increased levels of ERBB2 expression.

Expression levels of FOXP3 were examined in normal and cancerous human cell lines. The highest levels of expression were found in HMEC (normal human mammary epithelial cells); slightly lower levels were noted in the immortalized but nonmalignant cell line MCF-10A. Ten immortalized, malignant cancer lines were also tested for FOXP3 expression levels, and showed varying expression levels, all lower than in HMEC or MCF-10A cells, supporting the role of FOXP3 as a tumor suppressor. (Two cell lines lacked any detectable expression, and the other eight were reduced by 1.5-20 fold.) PCR and sequencing analysis

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Downregulation of HIF1A Expression by HuSH Constructs



HEK293T cells were cotransfected with pCMV-HIF1A cDNA together with four shRNA constructs against HIF1A. Western blot data demonstrates that three out of the four constructs significantly downregulate the cotransfected HIF1A expression. [More details can be found at www.origene.com/rna/](http://www.origene.com/rna/)

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showed that HMEC transcripts of FOXP3 were full-length, while MCF-10A cells showed expression of a transcript variant of FOXP3 lacking a single exon. None of the malignant cell lines expressing detectable FOXP3 expression contained a full-length transcript. Analysis of corresponding oncogene transcript levels showed that ERBB2 expression levels in these cell lines were higher in all ten cancer cell lines than in HMEC.

The researchers examined over 600 samples of breast cancer tissue, and showed significant downregulation of FOXP3 in those samples. Two common somatic mutants of FOXP3 identified in these cancer samples were used in promoter studies, and showed marked decreases in repressor activity on ERBB2. This decrease in repression was accompanied by increased levels of ERBB2 expression.

The initial work by the authors of this study demonstrated that a FOXP3 mutation significantly increases the risk of mammary carcinoma. Subsequent experiments implicate FOXP3 as a repressor of ERBB2 in human breast epithelial cells. Interestingly, FOXP3 is an X-linked gene, which is unusual for a tumor suppressor gene, as X-inactivation can markedly increase the potential for dysregulation of the corresponding oncogene.

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FOXP3 is an X-linked gene, which is unusual for a tumor suppressor gene.

TRUECLONE COLLECTION

Cat. No	Description
SC310403	Homo sapiens forkhead box P3 (FOXP3) as 10 ug transfection-ready DNA NM_014009.2 Cost: \$850 USD Delivery: Immediate
SC124018	OriGene unique variant 1 of Homo sapiens forkhead box P3 (FOXP3) as 10ug transfection-ready DNA NM_014009.2 Cost: \$850 USD Delivery: Immediate
SC128161	Homo sapiens v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) (ERBB2), transcript variant 1 as 10 ug transfection-ready DNA NM_004448.2 Cost: \$1550 USD Delivery: 3 days
SC301045	Homo sapiens v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) (ERBB2), transcript variant 2 as 10 ug transfection-ready DNA NM_001005862.1 Cost: \$2550 USD Delivery: 5-7 weeks
TR317157	HuSH 29mer shRNA Constructs against Homo sapiens FOXP3 Locus ID = 50943 Cost: \$595 USD Delivery: Immediate
TR513384	shRNA Constructs against Mus musculus FOXP3 Locus ID = 20371 Cost: \$595 USD Delivery: 3 weeks
TR320342	HuSH 29mer shRNA Constructs against Homo sapiens ERBB2 Locus ID = 2064 Cost: \$595 USD Delivery: Immediate
TR511550	shRNA Constructs against Mus musculus Erbb2 Locus ID = 13866 Cost: \$595 USD Delivery: 3 weeks
TA300035	Purified ERBB-2 Receptor Protein Tyrosine Kinase Rabbit anti-Human Polyclonal Antibody Cost: \$300 USD

TissueScan Tissue qPCR arrays—now there is more of a good thing!

Despite the incredible potential of cancer biomarkers, very few have been sufficiently validated to justify their use in developing drugs or making patient care decisions.

The number of cancer biomarkers in current use for clinical therapy is quite small. One of the major obstacles in biomarker validation is the difficulty in accessing a sufficient number of clinical samples with adequate documentation. Much promising, preliminary research ends after obtaining data from cancer cell lines or after microarray screening using a small number of paired cancer/normal tissues. With the availability of OriGene's TissueScan qPCR array containing over forty primary samples per plate, that preliminary research can now be extended with relevant human material.

TissueScan qPCR Arrays were invented to provide researchers with easy access to a large number of well-documented primary human biopsy specimens. OriGene Technologies, your gene company, teamed with Cytomyx, a distinguished biorepository, to introduce this new tool for gene expression studies in a wide variety of human cancers. Cytomyx is one of the largest sources of highly characterized human tissue samples in the world. It has over 140,000 biological specimens encompassing a wide range of human diseases. These samples have been used by world's leading pharmaceutical companies to identify new drug targets and investigate the role of genetics in human disease.

OriGene, with its expertise in cDNA preparation, converted the tissue samples into prenormalized cDNAs and arrayed them in 96-well qPCR plates. Each TissueScan panel contains 48 or 96 first-strand cDNAs prepared from individual tumor tissue blocks. Researchers who wish to validate a potential biomarker can simply design a pair of gene-specific primers and perform a qPCR experiment with any of these panels. A quick assay will generate a clean expression profile across 48-96 tumor samples in less than two hours.

Unlike microarrays that explore expression changes of multiple genes in a single tissue, TissueScan reveals the expression changes of a single gene in multiple tissues. Another way to think of TissueScan qPCR Array is as a "reverse microarray." This platform is especially useful for validation of a large number of leads obtained from a microarray experiment.

The Cancer Survey qPCR Array is a 96-well qPCR plate, representing 8 cancer types with 12 samples for each type.

Two types of TissueScan qPCR Arrays now available

In the past few issues of GeneDex, we have introduced TissueScan arrays that are specific to a single cancer type. Each of these panels contains samples of a specific tissue origin, such as prostate, breast, or colon. These highly popular arrays are ideal for the researchers that have a focused interest in a particular disease area. OriGene is continuously adding new arrays in this category, not only by expanding the types of tissues used in each array, but also by increasing the number of the samples available for each tissue type. We've added new panels with cDNA from liver cancer, sarcoma, melanoma, endometrial and gastroesophageal cancers, and increased the number of samples available for our breast, colon, and lung cancer panels.

As we continue to expand the TissueScan repertoire, we are acutely aware of the fact that cancer is a complex disease and to classify by tissue of origin might be an oversimplification. Key players in biological pathways (such as signal transduction, apoptosis or angiogenesis) are aberrantly expressed in cancers of multiple tissue origins. Researchers are often interested in scanning cancer samples derived from multiple tissue types, as genes with elevated expression in one cancer type might also be elevated in another. To meet this unmet need, OriGene launched the Cancer Survey qPCR Array.

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Continued from Page 11

The Cancer Survey qPCR Array is a 96-well qPCR plate, representing 8 cancer types with 12 samples for each type. Three samples from each cancer type are normal controls (surgical samples from the cancer patients defined by the pathologist as normal) and the remaining nine samples for each group represent the cancer progression stages. The layout of the Survey Array is shown in Table 1 (page 15).

As with all TissueScan qPCR Arrays, samples in the Cancer array panel are also normalized by beta-actin expression. Due to the differences in beta-actin expression between various tissues, the normalization is done within the tissue type, instead of across all tissues in the entire panel (see Figure 1 below).

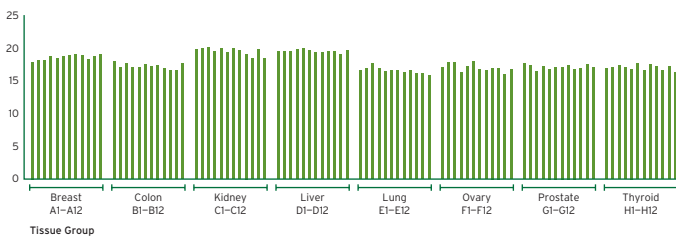


Figure 1. Beta-actin expression levels for samples in the Cancer Survey panel. The threshold cycle number (Ct value) is plotted as the Y-axis for all of the samples, which are detailed in Table 1. Briefly, samples from breast, colon, kidney, liver, lung, ovary, prostate, and thyroid tissues are grouped together and presented from left to right. Normalization of cDNA quantity using beta-actin levels was performed individually for each group, and then confirmed in the qPCR experiment whose results are presented here.

To validate the samples included in the survey panel, the expression pattern of survivin (a gene frequently overexpressed in many cancers) was determined by qPCR. Cancer samples from all tissue types showed higher survivin expression than normal tissues (see Figure 2 below).

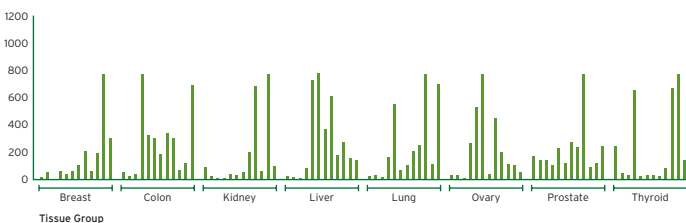


Figure 2. Expression levels of survivin for samples in the Cancer Survey Panel. Relative expression levels as determined by qPCR are presented for the samples from (left to right) breast, colon, kidney, liver, lung, ovary, prostate, and thyroid tissues.

The purpose of the Cancer Survey qPCR Array is to provide a quick overview of gene expression across the landscape of multiple cancer types. If one cancer appears to be most relevant for the candidate gene, subsequent research can be conducted with a disease-specific TissueScan qPCR Array, which provides a larger number of samples for each cancer type.

When you need to go beyond gene-expression pattern

TissueScan Tissue qPCR Arrays are an excellent tool to study the expression patterns of specific genes in cancer versus normal tissues. However, if complementary analysis is needed (such as immunohistochemistry, genotyping, analysis of the patient treatment history and outcome), additional information or other source material would be required. Thanks to OriGene's excellent relationship with the biorepository Cytomx, some related material (tissue, RNA, DNA, or protein) for many TissueScan samples can be acquired. Using the identification number for each sample (indicated in the pathology report, e.g. C1000015482), customers can request additional information and various related materials for many TissueScan samples.

EXTENSIVE CLINICAL DATA

As all TissueScan samples originate from the Cytomx tissue collection, they are accompanied by extensive clinical and pathology data available at the time the sample was originally collected. For about 50% of the samples ("linked samples"), Cytomx can obtain new data relevant to the donor, such as treatment outcomes. The clinical data team, operating strictly within the guidelines of HIPAA, is able to undertake new data retrieval projects for clients in order to identify samples that may be suitable for a project that has highly specific requirements unmet by the data obtained at the time of sample collection.

RELATED SOURCE PRODUCTS

RNA, DNA, protein and tissue sections are generated from a meticulously maintained, highly annotated tissue collection. Prior to RNA/DNA/protein extraction, the histology laboratory prepares an hemotoxylin and eosin (H&E) stained slide of each individual tissue sample. The H&E slide is then reviewed by a pathologist to determine an accurate sample level diagnosis and description of sample cellularity. Each RNA, DNA, or protein sample is supplied with an abstracted pathology report,

Continued on Page 14

Cancer/Normal Tissues cDNAs in qPCR Array Panels

Gene expression profile · SNP analysis Biomarker validation

TissueScan is a qPCR array panel for fast and accurate survey of gene expression levels or SNP profiles. Normalized cDNAs from 48-96 high-quality clinical tissues (provided by Cytomyx) are assembled into a single ready-to-use panel. Freed of the burden to procure human samples, researchers can focus on SNP analysis or validation of biomarkers, such as those obtained by microarray or differential display.

Convenient

Remove the hurdle of tissue procurement so you can focus on validation and discovery

Comprehensive

wide spectrum of cancer types;
includes normal and all progression stages

Reliable

Pathologist verified tissues with detailed pathology reports and histology images

Fast

Results in less than 2 hours

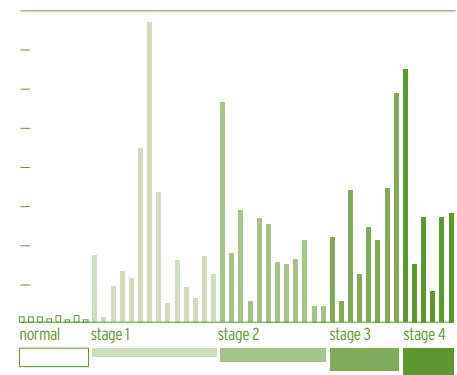
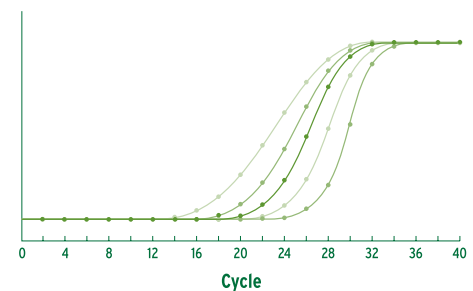
Currently Available

New panels released monthly

Prostate Cancer	Breast Cancer
Lung Cancer	Ovarian Cancer
Colon Cancer	Lymphoma
Thyroid Cancer	Kidney Cancer
Crohns and Colitis	Gastroesophageal Cancer
Melanoma	Liver Cancer
Multi-Cancer Survey Panel	

Also Available

Normal Human Brain
Normal Human Major Tissue



Scan results for topoisomerase II alpha with the Lung Cancer TissueScan Panel (HLRT101)



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Tissue, RNA, DNA, or protein corresponding to many TissueScan samples is now available.

digital H&E images captured at 4x and 20x magnification and a tabular presentation of additional patient data.

RNA

A proprietary set of standard operating procedures is utilized to extract RNA from frozen, OCT-embedded samples and to ensure accurate measurement of intact total RNA, free of contaminating DNA and protein. Please note that part of the RNA extraction protocol involves column purification which may remove small RNA species. RNA is supplied as a 1–2ug aliquot. A detailed QC data set is provided with each sample including:
Agilent Bioanalyzer 28S/18S ratio
Agilent Bioanalyzer Electropherogram
RT-PCR gel image

DNA

DNA samples are exclusively derived from frozen, OCT-embedded tissues, ensuring the reliable isolation of high quality genomic DNA suitable for use in a wide range of experiments such as SNP detection. Proprietary protocols are optimized for removal of contaminating protein and RNA. DNA is supplied as a 1–2ug aliquot. A detailed QC data set is provided with each sample including:
A260/A280 ratio
End-point PCR gel image

PROTEIN

Total protein lysates are also derived from frozen, OCT-embedded tissue samples. Lysates are generated using a modified RIPA buffer (lacking SDS) in the presence of protease and phosphatase inhibitors. Protein quantification is performed using the BCA Protein Assay. Total protein lysates are supplied as 250ug aliquots. A detailed QC data set is provided with each sample including:
PAGE gel image visualized by SYPRO Ruby (or Coomassie Blue)
Western blot image using a beta-actin monoclonal antibody

TISSUE SECTIONS

Tissue sections are an ideal solution for rapidly identifying and characterizing biomarkers of interest and can be used for applications such as immunohistochemistry (IHC), in-situ hybridization (ISH), laser capture microscopy (LCM) or even RNA/DNA/protein extractions. Prior to sectioning, all tissue blocks are pathologist reviewed and the cellularity of the sample is clearly described.

All slides are shipped complete with pathology verification data, clinical annotation (including patient age, gender, AJCC TNM data and minimum stage grouping), abstracted pathology reports and digital H&E images of the source block for both the FFPE and frozen formats. Tissue sections are supplied in sets of FIVE (5) slides, each cut to a thickness of 5 microns (5um) provided on SuperFrost Plus glass slides.

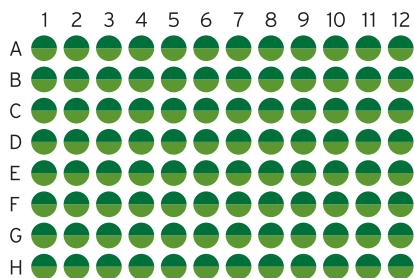
With the new Cancer Survey panel and the availability of related materials for TissueScan samples, we hope to provide a complete tool set for candidate biomarker researchers. With a biomarker in mind, researchers can fully investigate the expression pattern of the gene using the following procedure:

- Step 1** Use a Cancer Survey TissueScan qPCR Array to screen eight cancer types by qPCR and identify the disease for which your candidate marker is most relevant.
- Step 2** Use a disease-specific TissueScan qPCR Array to survey additional normal and cancer samples from the specified cancer type.
- Step 3** Obtain the RNA, DNA, Protein or Tissues of the relevant cancer sample(s) from the original tissue source for further investigation.

OriGene's TissueScan panels have quickly become an important tool for cancer researchers. With the addition of the Cancer Survey panel and the availability of related source materials for many TissueScan samples, the TissueScan product line should be your first choice for cancer biomarker studies.

You can see the complete list of TissueScan qPCR Arrays on our website at <http://www.origene.com/geneexpression>. For questions about TissueScan Oncology panels, please contact OriGene at 888-267-4436 (outside the US, call 301-340-3188) or write to us at techsupport@origene.com. For inquiries about related source material (RNA, DNA or protein) or additional sample information, please contact Cytomyx at 1-781-863-9720.

TABLE 1: CSRT101 AND CSRT501



Well	Cancer	Patient	Appearance	Stage
A01	Breast	Female-44	Normal	0
A02	Breast	Female-45	Normal	0
A03	Breast	Female-32	Normal	0
A04	Breast	Female-47	Tumor	I
A05	Breast	Female-52	Tumor	I
A06	Breast	Female-57	Tumor	IIA
A07	Breast	Female-42	Tumor	IIA
A08	Breast	Female-63	Tumor	IIIA
A09	Breast	Female-61	Tumor	IIIA
A10	Breast	Female-55	Tumor	IIIC
A11	Breast	Female-45	Tumor	IV
A12	Breast	Female-42	Tumor	IV
B01	Colon	Male-61	Normal	0
B02	Colon	Male-91	Normal	0
B03	Colon	Female-37	Normal	0
B04	Colon	Female-93	Tumor	I
B05	Colon	Male-65	Tumor	IIA
B06	Colon	Male-78	Tumor	IIA
B07	Colon	Male-66	Tumor	IIA
B08	Colon	Male-86	Tumor	III
B09	Colon	Female-42	Tumor	IIIB
B10	Colon	Male-61	Tumor	IIIC
B11	Colon	Male-63	Tumor	IIIC
B12	Colon	Female-51	Tumor	IV
C01	Kidney	Male-71	Normal	0
C02	Kidney	Male-66	Normal	0
C03	Kidney	Female-54	Normal	0
C04	Kidney	Male-52	Tumor	I
C05	Kidney	Female-55	Tumor	I
C06	Kidney	Male-52	Tumor	I
C07	Kidney	Female-57	Tumor	II
C08	Kidney	Male-59	Tumor	III
C09	Kidney	Male-37	Tumor	III
C10	Kidney	Male-64	Tumor	III
C11	Kidney	Male-70	Tumor	IV
C12	Kidney	Male-51	Tumor	IV
D01	Liver	Male-81	Normal	0
D02	Liver	Male-86	Normal	0
D03	Liver	Female-33	Normal	0
D04	Liver	Male-79	Tumor	I
D05	Liver	Female-58	Tumor	I
D06	Liver	Male-66	Tumor	I
D07	Liver	Female-63	Tumor	II
D08	Liver	Male-68	Tumor	II
D09	Liver	Female-62	Tumor	II
D10	Liver	Male-71	Tumor	IIIA
D11	Liver	Male-21	Tumor	IV
D12	Liver	Male-66	Tumor	IV

TissueScan Tissue qPCR Arrays are the fruit of a collaboration between the highly reputable tissue bank Cytomyx and OriGene's innovative molecular biology experts.

Well	Cancer	Patient	Appearance	Stage
E01	Lung	Female-49	Normal	0
E02	Lung	Male-79	Normal	0
E03	Lung	Female-62	Normal	0
E04	Lung	Male-71	Tumor	IA
E05	Lung	Male-64	Tumor	IB
E06	Lung	Female-85	Tumor	IB
E07	Lung	Male-72	Tumor	IB
E08	Lung	Male-63	Tumor	IIB
E09	Lung	Male-71	Tumor	IIB
E10	Lung	Male-58	Tumor	IIIA
E11	Lung	Male-80	Tumor	IIIB
E12	Lung	Male-51	Tumor	IV
F01	Ovarian	Female-70	Normal	0
F02	Ovarian	Female-31	Normal	0
F03	Ovarian	Female-42	Normal	0
F04	Ovarian	Female-29	Tumor	IA
F05	Ovarian	Female-43	Tumor	IB
F06	Ovarian	Female-51	Tumor	IC
F07	Ovarian	Female-80	Tumor	IIB
F08	Ovarian	Female-46	Tumor	IIIA
F09	Ovarian	Female-52	Tumor	IIIB
F10	Ovarian	Female-74	Tumor	IIIC
F11	Ovarian	Female-77	Tumor	IIIC
F12	Ovarian	Female-79	Tumor	IV
G01	Prostate	Male-68	Normal	0
G02	Prostate	Male-65	Normal	0
G03	Prostate	Male-76	Normal	0
G04	Prostate	Male-70	Lesion	I
G05	Prostate	Male-63	Lesion	II
G06	Prostate	Male-70	Lesion	II
G07	Prostate	Male-71	Lesion	II
G08	Prostate	Male-56	Lesion	II
G09	Prostate	Male-63	Tumor	II
G10	Prostate	Male-53	Lesion	III
G11	Prostate	Male-65	Lesion	III
G12	Prostate	Male-61	Tumor	III
H01	Thyroid	Female-30	Normal	0
H02	Thyroid	Female-68	Normal	0
H03	Thyroid	Female-46	Normal	0
H04	Thyroid	Female-15	Tumor	I
H05	Thyroid	Female-28	Tumor	I
H06	Thyroid	Female-39	Tumor	I
H07	Thyroid	Male-57	Tumor	II
H08	Thyroid	Male-74	Tumor	II
H09	Thyroid	Female-76	Tumor	III
H10	Thyroid	Female-52	Tumor	III
H11	Thyroid	Male-52	Tumor	IVA
H12	Thyroid	Female-45	Tumor	IVA

TrueClone Access Collection—keep your research options open!

OriGene's TrueClone collection of full-length human cDNA clones in mammalian expression vectors is a unique assembly rivaled by no other assortment commercially available. Over 24,000 unique cDNAs can be purchased as highly purified DNA ready for transfection. As a special thank-you to our customers, OriGene has designated 5000 of these expression-ready, full-length cDNA clones as the TrueClone Access™ Collection. Every clone in this collection is specially priced at \$95 USD for a 10 ug quantity. This enables single-target analysis as well as an affordable systems biology approach to high-throughput screening, functional studies, and protein production.

The Access Collection includes some of our most popularly ordered items, and includes transcription factors, protein kinases, transmembrane genes, proteases, G-protein coupled receptors, and many more gene family members (see Table 1 for details). A complete list of Access collection clones can be downloaded from the OriGene website at <http://www.origene.com/assets/Documents/Access/WebListing.xls>.

TABLE 1. THE ACCESS COLLECTION INCLUDES 5000 OF ORIGENE'S MOST POPULARLY ORDERED CLONES, AND REPRESENTS MANY GENE FAMILIES.

Gene Family	Number of Clones
Transcription Factors	661
Protein Kinase	143
GPCR	95
NHR	21
Ion Channels	67
P450	16
Phosphate	65
Protease	160
Transmembrane	902

As with all TrueClones, the clones in the Access Collection are human full-length cDNA clones whose expression is driven by the CMV promoter. The uniformity of the vector for all clones makes this collection ideal for high-throughput studies. And since the clones are shipped as 10 ug of purified plasmid DNA, they are available for immediate application; no transformation or DNA purification is required.

Access clones are shipped as 10 ug of purified plasmid DNA, so they are available for immediate application. No transformation or DNA purification is required.

The Access collection is available in two formats. You can purchase the clones individually or in bulk, and receive 10 ug of DNA per clone in individual tubes. Or you can bank the entire Access collection and receive 1 ug purified DNA plasmid for all 5000 clones, delivered as a 50 ng/uL solution arrayed in 96 well plates. This amount of purified DNA plasmid (1 ug) is sufficient for any number of applications, including PCR, probe synthesis, sequencing, transfection, and more. See Table 2 for possibilities.

TABLE 2. A NUMBER OF APPLICATIONS CAN BE PERFORMED WITH 1 UG OF PURIFIED DNA.

Number of Reactions	Application	Typical amount of DNA
100	PCR	10 ng
5	Transfection	200 ng
8	Sequencing Reactions	125 ng
2	Enzyme Digests/Subcloning	500 ng
1	In vitro Protein Synthesis	1 ug
40	Probe Synthesis	25 ng

At \$95 USD per clone, you can't afford not to take advantage of this unique collection. Visit OriGene's website today to learn if your gene of interest is something you can Access!



Keep Your Research Options Open

10µg purified plasmid for
\$95

5000 transfection-ready full-length cDNA clones. The options are endless.

Priced and packaged for cost-efficiency and convenience, the **TrueClone Access™ Collection** enables single-target analysis as well as an affordable systems biology approach to high-throughput screening, functional studies, and protein production.

The 5000 cDNA clones in the Access collection parallel full-length clones found in the Mammalian Gene Collection (MGC). Unlike MGC clones, all Access clones are housed in CMV vectors for easy adaptation to high throughput manipulation and are provided as 10µg of purified transfection-ready plasmid DNA.

Buy individually, in combination, or bank the entire collection. Your options are open at www.origene.com/access



TrueClone
access™
Collection

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300 Difficult to clone human full-length cDNAs just released

**Most not available elsewhere
Immediate delivery to your lab
Provided as 10ug Transfection-ready
DNA plasmid**

OriGene has recently added another 300 difficult-to-clone genes to the TrueClone collection, the most complete set of human full-length cDNA clones. Most of these newly added clones are unavailable from any other suppliers.

Some examples are:

Long Genes—MACF1 (18kb), SACS (13kb), PRKDC (12kb), ASPM (10.3kb), RELN (10kb), BRCA2 (10kb) and many more

Rare Genes—Notch1, Notch2, Notch3, Notch4

GC-Rich Genes—KCNQ1 (80% GC in the 5' region of the ORF)

Toxic Genes—SCN2A (cannot propagate in standard E. coli strains)

OriGene is dedicated to cloning every human full-length cDNA, no matter how daunting the task so you can focus on your research.

Full-length human cDNAs
Expression-ready in plasmids with pCMV promoters
Provided as 10 ug transfection-ready plasmid DNA

Call 1.888.267.4436 or write to techsupport@origene.com for further assistance.



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Citations

OriGene is proud of its customers and their track records of scholarly article publication. Here are some citations of recently published, peer-reviewed manuscripts from OriGene customers. Take a look, and see how an OriGene product may help your experiments blossom into a published research article!

TRUECLONE CDNA PLASMIDS

Am J Physiol Lung Cell Mol Physiol. 2007 Aug 24; [Epub ahead of print] **[PARP1]**

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Mol Biol Cell. 2007 Sep; 18(9):3486-501. **[CCDC91]**

Mol Cell Biol. 2007 Oct; 27(19):6669-85. **[DGKQ]**

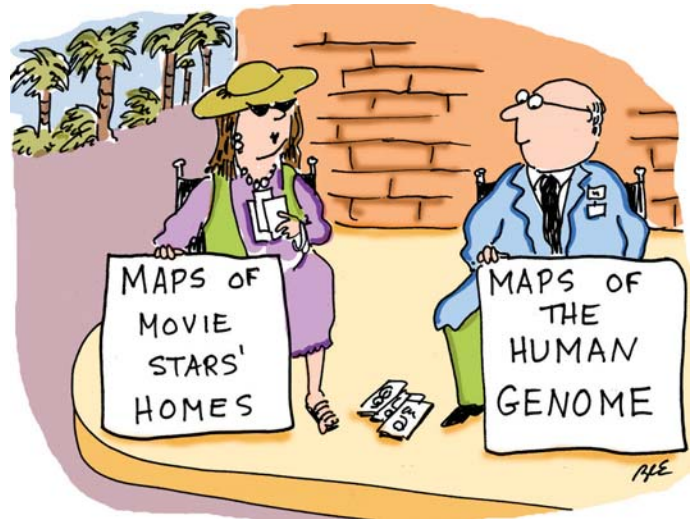
Mol Cell Proteomics. 2007 Sep 12; [Epub ahead of print] **[WBP2, NSFL1C]**

Mol Pharmacol. 2007 Aug 27; [Epub ahead of print] **[SLC10A1]**

Nucleic Acids Res. 2007; 35(16):e105. **[MYC]**

Nucleic Acids Res. 2007 Sep 28; [Epub ahead of print] **[DICER1 and XPO5]**

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J Neurosci. 2007 Aug 15;27(33):8866-76.

NORTHERN BLOTS

Am J Physiol Cell Physiol. 2007 Sep 26; [Epub ahead of print]

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conferences – check us out!

Neuroscience 2007

Nov 3–7, 2007 San Diego, CA; Booth #3517

The American Society for Cell Biology

Dec 1–5, 2007 Washington, DC; Booth #1147



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